low-field susceptibility measurements, are consistent with this possibility. These data suggest that up to ~50% of the magnetization at low temperatures may arise from $S = 1$ spin triplets, the remainder coming from $S = {}^1/_2$ doublets. Our recent ESR measurements do, in fact, conclusively demonstrate the occurrence and evolution of spin triplets with increasing doping level.[6]

In conclusion, we describe for the first time a synthetic method to introduce low-spin $TCNQF_4$ as molecular spacers in the solid state of $(HMT)_2$-$TCNQF_4$-$(AsF_{5.5})_y$ to separate high-spin HMT molecules into molecular domains that avoids the spin pairing between adjacent HMT radicals. This synthetic manipulation results in stabilization of the triplet state of dicationic HMT and ambient temperature stable organic solids possessing a high spin density on the order of 1.0-1.6 spins ${}^1/_2$ per formula unit with an anomalously small interspin coupling. Since stable ground-state high-spin organics are thought to be essential components in the design of organic ferromagnetic solids, the results suggest that $(HMT)_2$-$TCNQF_4$ may be a good model complex for the study of organic ferromagnets. Also the observation encourages us to study further the conditions necessary to achieve ferromagnetism in this type of organic material system.[8]

(8) Cowan, D. O.; Wiygul, F. M. *C&EN* July 21, 1986, 28-45.

Calibration of the Bicyclo[2.1.0]pent-2-yl Radical Ring Opening and an Oxygen Rebound Rate Constant for Cytochrome P-450†

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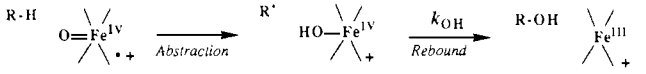
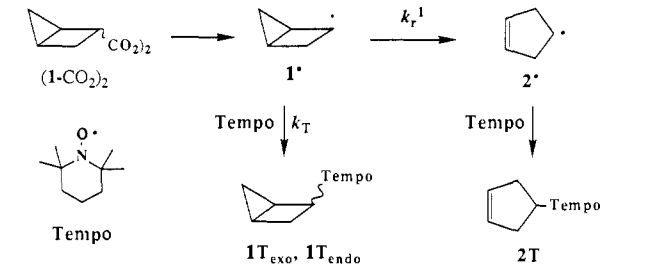
Cytochrome P-450 catalyzed oxidation (hydroxylation) of hydrocarbons[1] is believed to occur by a mechanism involving hydrogen atom abstraction from the substrate (R-H) followed by rapid transfer of HO• to the resulting alkyl radical (R•). This so-called *oxygen rebound mechanism*[1] is consistent with reports of allylic and stereo- and regiochemical scrambling and of large *intrinsic* or *intramolecular* deuterium kinetic isotope effects in P-450 oxidations. *Oxygen rebound* has supplanted an earlier concerted mechanism[2] which was proposed to account for the (then) preponderance of regio- and stereoselectivity and for the small *intermolecular* deuterium kinetic isotope effect.[1]

In an exciting and incisive investigation, Ortiz de Montellano and Stearns[3] have used the *radical-clock* method[4] to probe the mechanism and kinetics of the hydroxylation process at the in vivo temperature of 37 °C: methylcyclopropane, which would be hydroxylated via the cyclopropylmethyl (CPM) radical,[5,6] afforded only the unrearranged alcohol, viz. cyclopropanemethanol; bicyclo[2.1.0]pentane, on the other hand, afforded a mixture of both the corresponding unrearranged *and* rearranged alcohols in ca. 7:1 molar ratio. The latter result indicates that ring-opening of the intermediate bicyclo[2.1.0]pent-2-yl radical (**1**•) competes with the hydroxyl radical transfer or *rebound* step, and, since these processes are in direct competition, the implication[7-9] is that k_{OH}

Scheme I



Scheme II



Pseudo-first-order Kinetic Equation: $k_r^1/k_T = [\text{Tempo}].[2T]/([1T_{exo}]+[1T_{endo}])$

$\approx 7k_r^1 \gg k_r^{CPM}$. We report herein the first measurement of k_r^1 which, when combined with Ortiz de Montellano's P-450 data, provides the first estimate of k_{OH} for this species.

Both EPR spectroscopy[10-12] and Ortiz de Montellano's P-450 work[3] show that the rearrangement of **1**• → **2**• must be extremely rapid relative to the cyclopropylmethyl ring-opening, and only a chemical trapping procedure using a superbly efficient trap for carbon radicals seemed likely to provide a reliable value for k_r^1. Various considerations, including in particular the fact that trapping rate constants have been reliably measured and been found to be almost diffusion controlled,[13,14] do not depend significantly on alkyl radical structure,[6,13,14] and the fact that at 37 °C the trapping agent could, if necessary, be used as the neat liquid (6 M) led us to choose Tempo as our trap. Typically, the neat diacyl peroxide[15,16] $(1\text{-}CO_2)_2$ was added to a stirred, preheated (37 °C) solution of Tempo (30-fold excess) in chlorobenzene (for 1-5 M Tempo) or 2,2,4-trimethylpentane (<1 M Tempo). Nitroxide-induced decomposition[18] of the peroxide afforded reaction

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‡ NRCC Research Associate, 1988-89.

(1) For a detailed discussion and leading reference, see: Ortiz de Montellano, P. R. In *Cytochrome P-450: Structure, Mechanism, and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum: New York, 1986; pp 217-271. See, also: McMurry, T. J.; Groves, J. T. on pp 3-5.

(2) Hamberg, M.; Bjorkhem, I. *J. Biol. Chem.* **1971**, *246*, 7411-7416.

(3) Ortiz de Montellano, P. R.; Stearns, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 3415-3420.

(4) Griller, D.; Ingold, K. U. *Acc. Chem. Res.* **1980**, *13*, 317-323.

(5) Cyclopropylmethyl radical ring opens with a rate constant[6] $k_r^{CPM} = 1.2 \times 10^8\ M^{-1}\ s^{-1}$ at 37 °C.

(6) Beckwith, A. L. J.; Bowry, V. W.; Moad, G. *J. Org. Chem.* **1988**, *53*, 1632-1641.

(7) A word of caution: in making use of the alcohol product ratios to calculate k_{OH} we have assumed that rearranged radicals are efficiently converted into alcohols. However, many species which rearrange during hydroxylation are known to destroy the P-450 catalyst, presumably without formation of the alcohol.[8] The (kinetic) effect of this and of other possible non-alcohol-forming reactions[9] of the rearranged substrate radical may need to be investigated.

(8) Catalyst turnover vs P-450 destruction data[3] indicate that this would only have a minor effect on kinetic data for bicyclopentane since the rearranged alcohol is formed about six times more rapidly than P-450 destruction. On the other hand, if one assumes **1**• and CPM have the same *rebound* rate constant, viz. $2 \times 10^{10}\ s^{-1}$, one may readily calculate that 3-buten-1-ol *should* be formed at about the same rate that P-450 is being destroyed by cyclopropylmethane!

(9) For example, there is evidence for radical diffusion from model catalysts, see: Groves, J. T.; Nemo, T. E. *J. Am. Chem. Soc.* **1983**, *105*, 6243-6248. However, this is less likely for P-450.

(10) Whereas CPM may be observed by EPR at temperatures up to -120 °C,[11] Jamieson et al.[12] found that **1**• had completely rearranged to 3-cyclopentenyl radical, **2**, even as low as -160 °C, which indicates that $k_r^1 \gg k_r^{CPM}$ at low temperature. From the experimental conditions the authors estimated $k_r^1 \geq 10^2\ s^{-1}$ at -160 °C and $E_A \leq 5.7$ kcal/mol with log $A = 13.0$ ($\therefore k_r^1 \geq 10^9\ s^{-1}$ at 37 °C).

(11) Maillard, B.; Forrest, D.; Ingold, K. U. *J. Am. Chem. Soc.* **1978**, *98*, 7024-7026.

(12) Jamieson, C.; Walton, J. C.; Ingold, K. U. *J. Chem. Soc., Perkin Trans. 2* **1980**, 1366-1371.

(13) Radical clock calibrations[6] indicate $k_T^{1°\text{-alkyl}} \approx k_T^{2°\text{-alkyl}}$, and time-resolved radical quenching data[14] indicate that even 3°-alkyl and benzylic radicals are trapped nearly as rapidly.

(14) Chateauneuf, J.; Lusztyk, J.; Ingold, K. U. *J. Org. Chem.* **1988**, *53*, 1629-1632.

(15) Bis(bicyclo[2.1.0]pentane-2-carbonyl) peroxide $(1\text{-}CO_2)_2$ and bis(cyclopent-3-enecarbonyl) peroxide $(2\text{-}CO_2)_2$ were prepared by standard procedures[6] from the corresponding acids.[16,17]

(16) Brooks, P. R.; Brophy, B. V.; Bernard, V. *J. Chem. Soc., Perkin Trans 1* **1985**, 2509-2513.

(17) Cremer, S. E.; Blankenship, C. *J. Org. Chem.* **1982**, *47*, 1629-1632.

(18) Moad, G.; Rizzardo, E.; Soloman, D. H. *Tetrahedron Lett.* **1981**, *22*, 1165-1168.

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mixtures consistent with the scheme: Tempo + $(1\text{-}CO_2)_2 \rightarrow 1^\bullet + CO_2 + 1\text{-}CO_2H$ + Tempo(-H).

Coupled reversed-phase HPLC-MS gave satisfactory resolution of the alkoxyamine components, viz. $1T_{exo}$, **2T**, and $1T_{endo}$, in order of elution.[19] The components were identified from (a) HPLC retention times and mass spectra, (b) the "kinetic" relationship between their relative yields, i.e., $(1T_{exo} + 1T_{endo})/2T\alpha$[Tempo], and (c) co-injection of authentic **2T** made by treatment of $(2\text{-}CO_2)_2$[15,17] with Tempo. The major unrearranged radical product was presumed to be the exo isomer because trapping at the exo face of **1**• would clearly entail less nonbonded interaction (with the cyclopropyl CH_2 group on **1**•) than trapping at the endo face. This assignment is supported by the reversed-phase HPLC elution order ($1T_{exo}$ before $1T_{endo}$) since the polar N-O group would be more shielded from the solvent[19] in $1T_{endo}$ than in $1T_{exo}$.

Kinetic data, available as Supplementary Material, are consistent with relative rate constants of $k_r^1/k_T = 4.5 \pm 0.5$ M in chlorobenzene and 1.6 ± 0.2 M in 2,2,4-trimethylpentane, both at 37 °C. Radical clock[6,20] and laser flash photolysis measurements[14,21] of k_T indicate that this difference in k_r^1/k_T is due to the solvent dependence of the radical-trapping reaction, i.e., k_T^{alkyl} is about twice as large in alkanes as in aromatic solvents of similar molar mass.

We can assume that **1**• will be trapped by Tempo with essentially the same rate constant as a primary alkyl radical,[6,13,14] viz. $k_T = 1.5 \times 10^9$ M^{-1} s^{-1} in 2,2,4-trimethylpentane at 37 °C,[22] and hence the above kinetic data give $k_r^1 = 2.4 \times 10^9$ s^{-1}.[23-25] The 20-fold increase in the rate of rearrangement of **1**• relative to the rearrangement of cyclopropylmethyl[5] reflects the large amount of strain in the inner-ring bond[26] which is partly offset by an unfavorable orbital overlap for fission of this bond.[26] With $k_r^1 = 2.4 \times 10^9$ s^{-1} at 37 °C and an assumed preexponential of 10^{13} s^{-1}, we estimate the activation energy $E_A = 5.1$ kcal/mol and $k_r^1 = 1.7 \times 10^3$ s^{-1} at −160 °C, which is fully consistent with EPR data.[10-12]

The product data of Ortiz de Montellano and Stearns[3] suggest that *oxygen rebound* occurs about seven times faster than the rearrangement,[7] **1**• → **2**•, and, if we assume that binding of **1**• to the enzyme has little effect on k_r^1,[27,28] we calculate that the *oxygen rebound* rate constant $k_{OH} \approx 7k_r^1 \approx 2 \times 10^{10}$ s^{-1}. Thus *rebound* occurs more rapidly than many typical conformational and configurational changes and also perhaps more rapidly than the gross molecular motion of many enzyme-bound substrates. The numerous reported cases of retention of configuration and stereochemistry and, in particular, the remarkable regioselectivity observed in the hydroxylation of bicyclo[2.1.0]pentane (which undergoes *abstraction and rebound* only at the endo face[3]) are therefore readily explained.

In conclusion, calibration of the radical clock, **1**• → **2**•, suggests that *oxygen rebound* in cytochrome P-450 occurs with an effective rate constant of 2×10^{10} s^{-1} for this substrate/P-450 combination; it would, of course, be interesting to see whether other suitable clock substrates afford similar *rebound* rate constants. Preliminary microsome experiments with 1,1,2,2-tetramethylcyclopropane, following the methodology of Ortiz de Montellano et al.,[3] have yielded mixtures of the corresponding unrearranged and rearranged alcohols. Thus, this substrate may provide a valuable alternative clock for timing the kinetics of *oxygen rebound*. Further work in this area is in progress and will be the subject of a full paper.

Acknowledgment. We thank L. Hughes for preparing $1\text{-}CO_2H$, D. A. Lindsay for his technical assistance, and D. O. Foster for preparing the hepatic microsomes.

Registry No. 1, 185-94-4; **1**•, 84592-00-7; Tempo, 2564-83-2; P-450, 9035-51-2.

Supplementary Material Available: Table I listing relative hydroxylamine yields and relative rate constants vs [Tempo] in chlorobenzene and in 2,2,4-trimethylpentane (1 page). Ordering information is given on any current masthead page.

(19) 200 × 2 mm ODS column eluted with 77% CH_3CN/H_2O (0.005 M NH_4OAc) at 1.0 mL/min.

(20) Bowry, V. W. Ph.D. Dissertation, Australian National University, 1988.

(21) Bowry, V. W., unpublished laser flash photolysis data.

(22) From log $k_T^{nonyl} = 10.4 - 1.8/\theta$, where $\theta = 2.3RT$ kcal/mol.[14]

(23) This calculation implicitly assumes that the only reaction between **1**• and Tempo is coupling to produce $1T_{exo}$ and $1T_{endo}$. To our knowledge, this is the fastest calibrated alkyl radical rearrangement which involves bond breaking and making. Only the inversions of cyclopropyl[24] and certain 1-substituted cyclopropyl radicals are faster (e.g., for 1-methylcyclopropyl, $k_i = 8.2 \times 10^{10}$ s^{-1} at 37 °C).[25]

(24) Johnston, L. J.; Ingold, K. U. *J. Am. Chem. Soc.* **1986**, *108*, 2343-2348.

(25) Deycard, S.; Hughes, L.; Lusztyk, J.; Ingold, K. U. *J. Am. Chem. Soc.* **1987**, *109*, 2343-2348.

(26) Roberts, C.; Walton, J. C. *J. Chem. Soc., Perkin Trans. 2* **1983**, 879-885.

(27) Ring opening of **1** would require only a small amount of solvent/enzyme displacement since (a) the *internal* bond is broken and (b) an early transition state is suggested by the large heat of reaction. Furthermore, there is some evidence (e.g., the lack of substrate specificity in this form of P-450[3] and a substrate binding study for the functionally related LM_2 form[28]) that small hydrocarbon substrates are only loosely bound by hydrophobic forces in relatively capacious binding sites.

(28) Miwa, G. T.; Lu, A. Y. H. In *Cytochrome P-450: Structure, Mechanism, and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum: New York, 1986; pp 77-84. See, also: Ortiz de Montellano, P. R. *Acc. Chem. Res.* **1987**, *20*, 289-294.

Alkaline Phosphatase Catalyzes the Hydrolysis of Glucose 6-Phosphate via a Dissociative Mechanism

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E. coli alkaline phosphatase catalyzes a multistep reaction in which the hydrolysis of phosphate esters involves a phosphoryl enzyme intermediate. There appear to be two limiting mechanisms by which this intermediate could be formed. These are (1) an associative mechanism in the transition state of which there would be single bonds between the nonbridge oxygens and phosphorus and considerable bond order along the reaction coordinate and (2) a dissociative mechanism in the transition state of which the bond order between the phosphorus and the nonbridge oxygens would be above 1.4, and the bond order along the reaction coordinate would be very low. In the latter case the reaction could proceed via a free metaphosphate intermediate or, more likely, involve an expanded transition state. One way to distinguish between these mechanisms is to determine secondary ^{18}O isotope effects resulting from ^{18}O substitution in the nonbridge oxygens of a phosphate ester. An associative mechanism would show a sizeable normal isotope effect, since the bond order decreases to unity in the transition state, while a dissociative mechanism should show no isotope effect or a slightly inverse one, depending on the degree to which increased P-O bond order for the nonbridge oxygens balances loss of bending O-P-O vibrations in the transition state.[1]

To this end we have measured secondary ^{18}O kinetic isotope effects resulting from ^{18}O substitution in the three nonbridge oxygens on the alkaline phosphatase catalyzed hydrolysis of glucose 6-phosphate at 25 °C. Measurements were made at pH 8, which is on the pH optimum, and at pH 6, which is below the pK of the V/K profile where the chemistry is presumably rate limiting.[2] ^{18}O isotope effects were measured by the remote label method[3] in which 1-[^{13}C]glucose 6-phosphate-[$^{18}O_3$] was mixed with 1-[^{12}C]glucose 6-phosphate to give material with close to natural abundance of ^{13}C at C-1.[4] Since there is presumably no isotope

(1) Weiss, P. M.; Knight, W. B.; Cleland, W. W. *J. Am. Chem. Soc.* **1986**, *108*, 2761.

(2) Krishnaswamy, M.; Kenkare, U. W. *J. Biol. Chem.* **1979**, *245*, 3956.

(3) O'Leary, M. H.; Marlier, J. F. *J. Am. Chem. Soc.* **1979**, *101*, 3300.

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